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Sphingomonas ginsenosidimutans sp. nov., with Ginsenoside Converting Activity

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The Gram-reaction-negative, strictly aerobic, non-motile, non-spore-forming, and rod-shaped bacterial strain designated Gsoil 1429^T was isolated from the soil of ginseng cultivating field of Pocheon province in South Korea. This bacterium was characterized in order to determine its taxonomic position by using the polyphasic approach. Strain Gsoil 1429^T grew well at 25-37°C and at pH 7.0 on R2A and nutrient agar without NaCl supplement. Strain Gsoil 1429^T had *β*-glucosidase activity, which was responsible for its ability to transform ginsenoside Rb_1 (one of the dominant active components of ginseng) to F_2 via gypenoside XVII. On the basis of 16S rRNA gene sequence similarity, strain Gsoil 1429^T was shown to belong to the family *Sphingomonadaceae* and to be related to *Sphingomonas yunnanensis* YIM 003^T (98.2% sequence similarity), *S. phyllosphaerae* FA2^T (97.5%), *S. koreensis* JSS26^T (97.3%), and *S. asaccharolytica* IFO 15499^T (97.1%). The G+C content of the genomic DNA was 65.6%. The major respiratory quinone was Q-10 and the major fatty acids were summed feature 8 (comprising $C_{18:1}$ ω7c/ω9t/ω12t), $C_{16:0}$ and $C_{14:0}$ 2OH. DNA and chemotaxonomic data supported the affiliation of strain Gsoil 1429^T to the genus *Sphingomonas*. The DNA-DNA relatedness values between strain Gsoil 1429^T and its closest phylogenetically neighbours were below 28%. Strain Gsoil 1429^T could be differentiated genotypically and phenotypically from the recognized species of the genus *Sphingomonas*. The isolate therefore represents a novel species, for which the name *Sphingomonas ginsenosidimutans* sp. nov. is proposed, with the type strain Gsoil 1429^T (=KACC 14949^T =JCM 17074^T =LMG 25799^T).

***Keywords*:** 16S rRNA gene, polyphasic taxonomy, *S. ginsenosidimutans*, ginsenoside

Ginseng saponins (ginsenosides) have been recognized as being responsible for the biological and pharmacological activities of ginseng (Choi, 2008). More than 40 ginsenosides have been isolated from ginseng roots, with six major ginsenosides (ginsenosides Rb_1, Rb_2, Rc, Rd, Re, and Rg_1) constituting more than 90% of all ginsenosides (Park, 2004). Generally, the efficacy of ginsenoside increases with the extent of deglycosylation, which enhances its hydrophobicity and cell wall permeability. Biotransformation of ginsenoside (deglycosylation) can be achieved by hydrolyzing and removing a sugar moiety from the major ginsenosides using bacterial and fungal strains (Kim *et al*., 2005; Zhao *et al*., 2009; Park *et al*., 2010).

During the course of a study to screen ginsenoside converting aerobic bacterial strains in the soil of a ginseng cultivation field of Pocheon province, South Korea, several novel bacterial strains showing ginsenoside converting activity were screened. Among them, a strain designated Gsoil 1429^T, which could convert ginsenoside Rb_1 to F_2 via gypenoside XVII appeared to be a member of the genus *Sphingomonas* and became the subject of a taxonomic investigation.

The genus *Sphingomonas* was created by Yabuuchi *et al*. (1990) in order to accommodate Gram-negative, strictly aerobic, chemoheterotrophic, yellow-pigmented and rod-shaped bacteria that harbor sphingoglycolipids as cell envelope components. At the time of writing, the genus consisted of 45 validly named species, with *S. paucimobilis* as the type species (Euzéby, 1997).

In the present study, we conducted a phylogenetic (16S rRNA gene), phenotypic, genotypic, and chemotaxonomic analyses to determine the precise taxonomic position of this strain. On the basis of the results obtained in this study, we propose that strain Gsoil 1429^T should be placed in the genus *Sphingomonas* as the type strain of a novel species, *Sphingomonas ginsenosidimutans* sp. nov.

Materials and Methods

Isolation of bacterial strain and culture condition

Strain Gsoil 1429^T was originally isolated from soil of a ginseng field of Pocheon province in South Korea. This soil sample was thoroughly suspended in 50 mM phosphate buffer (pH 7.0) and spread on one-fifth strength modified-R2A (MR2A) agar plates as described by Im *et al*. (2010). The plates were incubated at 30°C for 1 month. Single colonies on the plates were purified by transferring them onto new plate either with modified-R2A or nutrient agar (Difco, USA). One isolate, Gsoil 1429^T, was cultured routinely on R2A agar or NA at 25°C and preserved as a suspension in nutrient broth with (20%, w/v) glycerol at -70°C. The strain Gsoil 1429^T was deposited to the Korean Agricultural Culture Collection (=KACC 14949^T), Japan Collection of Microorganisms (=JCM 17074^T) and the Belgian Co-ordinated Collections of Micro-organisms/Laboratorium voor Microbiologie (=LMG 25799^T).

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Phenotypic and biochemical characteristics

The Gram reaction was determined using the non-staining method, as described by Buck (1982). Cell morphology and motility was observed under a Nikon light microscope at ×1,000, with cells grown on R2A agar for 2 days at 30°C. Catalase and oxidase tests were performed as outlined by Cappuccino and Sherman (2002). Anaerobic growth was determined in serum bottles containing R2A broth supplemented with thioglycolate (1 g/L), in which the upper air layer had been replaced with nitrogen. In addition, biochemical phenotypic test were carried out using API 20NE, API ID 32GN, and API ZYM test kits according to the instructions of the manufacturer (bioMérieux, France). Tests for degradation of DNA (using DNase agar from Scharlau, with DNase activity by flooding plates with 1 M HCl), casein, starch, pullulan, and laminarin (Atlas, 1993) were performed and evaluated after 7 days. Growth at different temperatures (4, 10, 18, 30, 37, 42, and 45°C) and various pH values (pH 4.5-10.0 at intervals of 0.5 pH units) was assessed after 7 days of incubation. Salt tolerance was tested on R2A medium supplemented with 1-10% (w/v at intervals of 1% unit) NaCl after 7 days of incubation. Growth on nutrient agar, trypticase soy agar (TSA, Difco), and MacConkey agar (Difco) was also evaluated at 30°C.

Biotransformation of ginsenosides

Ginsenosides Rb_1, Rc, Rd, F_2, and compound K were purchased from Dalian Green Bio Ltd (Dalian, China). Ginsenosides gypenoside XVII and compound Mc_1 were obtained as described by An *et al*. (2010). The reaction mixture, containing 200 μl of 1 mM ginsenosides (Rb_1 and Rc, respectively) and 200 μl of a bacterial suspension inoculated in a nutrient broth, was incubated for 4 days, at 150 rpm and 30°C. During the reaction, a 50 μl aliquot was taken daily, extracted with an equal volume of water-saturated *n*-butanol, and subjected to TLC analysis. TLC was performed using $60F_{254}$ silica gel plates (Merck, Germany) with $CHCl_3$-CH_3OH-H_2O (65:35:10, v/v, lower phase) as the solvent. The spots on the TLC plates were detected by spraying with 10 % (v/v) H_2SO_4 followed by heating at 110°C for 5 min.

PCR amplification, 16S rRNA gene sequencing, and phylogenetic analysis

The genomic DNA of strain Gsoil 1429^T was extracted using a commercial genomic DNA-extraction kit (Solgent, Korea). The 16S rRNA gene was amplified from the chromosomal DNA using the universal bacterial primer pair 9F and 1512R and the purified PCR products were sequenced by Solgent Co. Ltd (Daejeon, Korea) (Im *et al.*, 2010). Full sequences of the 16S rRNA gene were compiled using SeqMan software (DNASTAR, USA). The 16S rRNA gene sequences of related taxa were obtained from GenBank and EzTaxon server (Chun *et al.*, 2007). Multiple alignments were performed by Clustal_X program (Thompson *et al*., 1997) and gaps were edited in the BioEdit program (Hall, 1999). Evolutionary distances were calculated using the Kimura two-parameter model (Kimura, 1983). The phylogenetic trees were constructed by using the neighbor-joining (Saitou and Nei, 1987) and the maximum-parsimony (Fitch, 1971) methods with the MEGA4 Program (Kumar *et al.*, 2008) with bootstrap values based on 1,000 replications (Felsenstein, 1985).

Isoprenoid quinones, cellular fatty acids, and polar lipid analysis

Isoprenoid quinones were extracted with chloroform/methanol (2:1, v/v), evaporated under a vacuum and re-extracted in *n*-hexane-water (1:1, v/v). The crude quinone in *n*-hexane solution was purified using Sep-Pak Vac Cartridges Silica (Waters) and subsequently analyzed by HPLC, as described by Hiraishi *et al*. (1996). Cellular fatty acid profiles were determined for strains grown on R2A agar for 48 h at 30°C. The cellular fatty acids were saponified, methylated, and extracted according to the protocol of the Sherlock Microbial Identification System (MIDI). The fatty acid methyl esters were then analyzed by gas chromatography (model 6890; Hewlett Packard) using the Microbial Identification software package (Sasser, 1990). Polar lipids were extracted for freeze-dried cells grown on R2A broth for 48 h at 30°C, and examined by two-dimensional TLC and identified as described by Minnikin *et al.* (1977).

Determination of DNA G+C content

For measurement of the G+C content of chromosomal DNA, the genomic DNA of strain Gsoil 1429^T was extracted and purified as described by Moore and Dowhan (1995) and enzymatically degraded into nucleosides. The G+C content was then determined as described by Mesbah *et al.* (1989), using reverse-phase HPLC.

DNA-DNA hybridization

DNA-DNA hybridization experiments were performed between strain Gsoil 1429^T, and 4 reference strains (*S. yunnanensis* YIM 003^T, *S. phyllosphaerae* FA2^T, *S. koreensis* JSS26^T, and *S. asaccharolytica* IFO 15499^T) with the method described by Ezaki *et al*. (1989) using photobiotin-labeled DNA probes and micro-dilution wells. Hybridization was performed at 51°C with five replications for each sample. The highest and lowest values obtained for each sample were excluded and the means of the remaining three values were converted to percentage DNA-DNA relatedness values.

Results and Discussion

Morphological and phenotypic characteristics

Cells of strain Gsoil 1429^T were Gram-reaction-negative, strictly aerobic, non-spore-forming, non-motile, rod shaped, oxidase-positive and catalase-positive. The colonies grown on R2A agar plates (Difco) for 2 days were smooth, circular, yellowish in color, convex, and 2-3 mm in diameter. On R2A agar, Gsoil 1429^T was able to grow at 18-42°C, but not at 10°C and 45°C. The isolate grew on nutrient agar and TSA, but not on MacConkey agar. The phenotypic and chemotaxonomic characteristics that differentiate the strain Gsoil 1429^T from other *Sphingomonas* species are listed in Table 1.

Biotransformation of ginsenosides

A time course study of the biotransformation of the ginsenoside Rb_1 was conducted. The TLC results are shown in Fig. 1. Ginsenoside Rb_1 was transformed into two types of metabolites: F_2 via gypenoside XVII (line 6), of which the Rf value was slightly lower than that of ginsenoside Rd. Ginsenoside Rc was transformed into compound Mc_1 (line 7), of which the Rf value was slightly above that of ginsenoside Rd. As shown in Fig. 1, ginsenoside Rb_1 was almost completely hydrolyzed after 3 days, where the levels of F_2 and compound Mc_1 reached maximums after 4 days.

Phylogenetic analysis

The 16S rRNA gene sequences of the strain Gsoil 1429^T determined in this study were continuous stretches of 1,439 bp (base position 20-1512 with respect to the *Escherichia coli*

Table 1. Differentiating characteristics of *Sphingomonas ginsenosidimutans* Gsoil 1429^T and the type strains of related *Sphingomonas* species.
Strains: 1, *Sphingomonas ginsenosidimutans* Gsoil 1429^T; 2, *Sphingomonas yunnanensis* YIM 003^T; 3, *Sphingomonas phyllosphaerae* FA2^T; 4, *Sphingomonas koreensis* JSS26^T; 5, *Sphingomonas asaccharolytica* IFO 15499^T.
All data from this study, except the DNA G+C contents and polar lipids of the reference strains (taken from Takeuchi *et al*., 1995; Lee *et al*., 2001; Rivas *et al*., 2004; Zhang *et al*., 2005). All strains were Gram-reaction-negative, yellowish rods, positive for catalase and oxidase activities, but negative for hydrolysis of starch and casein. In API 20 NE and API ID 32 GN kits, all strains were positive for arginine dihydrolase, urease and assimilation of D-glucose. All strains were negative for nitrate reduction, indole production, acid production from glucose, hydrolysis of gelatin, and assimilation of the following substrates: D-mannitol, gluconate, caprate, phenyl-acetate, D-sorbitol, 2-ketogluconate, 4-hydroxybenzoate, D-ribose, inositol, itaconate, suberate, acetate, lactate, 5-ketogluconate and 3-hydroxybenzoate. In API ZYM kits, all the strains were positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, α-glucosidase, and β-glucosidase activities. +, Positive; -, negative; ND, no data available.

Characteristic	1	2	3	4	5
Motility	-	+	+	+	+
Growth temperature (°C)	18-42	4-37	10 30	4-45	10-42
Salt tolerance at 1% (w/v)	-	+	+	+	+
Oxidase	+	+	-	+	+
Catalase	+	+	+	+	-
Anaerobic growth	-	-	-	+	-
Hydrolysis of					
DNA	+	-	-	-	+
Starch	-	-	-	+	-
Pullulan	+	-	+	-	+
Laminarin	+	-	+	+	+
Carbon utilization of					
L-Arabinose	-	+	+	-	+
D-Mannose	+	-	+	-	+
D-Maltose	-	+	+	+	+
Adipate	+	-	-	-	-
Malate	+	+	+	+	-
Citrate	-	+	+	+	-
Salicin	-	+	+	+	-
D-Melibiose	+	+	+	+	-
L-Fucose	+	-	-	-	-
Propionate	+	-	-	+	-
Valerate	-	-	-	+	-
L-Histidine	-	+	-	+	-
3-Hydroxybutyrate	+	-	-	+	-
L-Proline	+	-	+	+	-
L-Rhamnose	-	-	+	-	-
N-Acetyl-D-glucosamine	+	+	+	+	-
D-Sucrose	+	-	+	+	-
Malonate	+	-	-	-	-
L-Alanine	-	-	+	-	-
Glycogen	+	-	+	-	+
L-Serine	-	-	-	+	-
API ZYM test results					
Lipase (C14)	-	+	+	-	-
Cystine arylamidase	+	-	+	+	+
α-Chymotrypsin	+	-	-	+	-
α-Galactosidase	+	+	+	-	+
β-Galactosidase	-	+	+	-	+
β-Glucuronidase	-	+	+	-	+
N-Acetyl-β-glucosaminidase	+	+	+	+	-
α-Mannosidase	-	+	+	-	-
α-Fucosidase	-	+	+	-	-
Predominant polar lipids[a]	PE, PG, SGL, PC DPG	PE, PG, DPG, PC, SGL, PL	PE, PG, DPG, PC, SGL, PL1, PL2, PME, PDE	ND	ND
G+C content (mol%)	65.6	67.5	61	66	64.8

[a] Abbreviations: DPG, diphosphatidylglycerol; PC, phosphatidylcholine; PDE, phosphatidyldimethylethanolamine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PL, PL1, PL2, unidentified phospholipids; PME, phosphatidylmonomethylethanolamine; SGL, sphingoglycolipid.

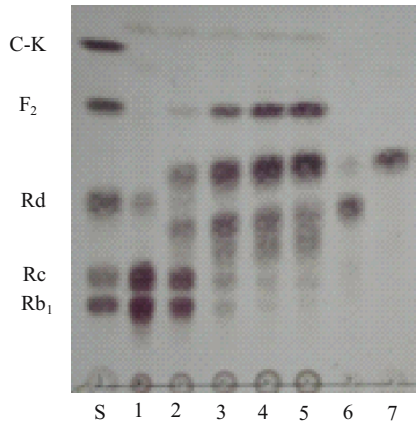

Fig. 1. TLC analyses of time-course transformation of ginsenoside Rb_1 and Rc by strain Gsoil 1429^T Developing solvent: $CHCl_3$/MeOH/H_2O (65:35:10, v/v, lower phase). S, saponin standards; 1, mixture of Rb_1 and Rc; 2, 1 day; 3, 2 days; 4, 3 days; 5, 4 days; 6, gypenoside XVII; 7, compound Mc_1. Abbreviations: C-K, compound K.

numbering system), which were deposited in a GenBank database (accession number HM204925). A sequence similarity calculation from using the EzTaxon server [http://www.eztaxon.org/; Chun *et al*. (2007)] indicated that the closest relatives of strain Gsoil 1429^T were *Sphingomonas yunnanensis* YIM 003^T (98.2%), *S. phyllosphaerae* FA2^T (97.5%), *S. koreensis* JSS26^T (97.3%), and *S. asaccharolytica* IFO 15499^T (97.1%). This relationship between strain Gsoil 1429^T and other members of the genus *Sphingomonas* was also evident in the phylogenetic tree, which used over 1400 nt (Fig. 2). Strain Gsoil 1429^T, *S. yunnanensis* YIM 003^T, and *S. phyllosphaerae* FA2^T formed a monophyletic group with a bootstrap high value (97%), which was supported by both tree making methods used in this study.

Cellular fatty acid, quinine, and polar lipid composition

The major respiratory quinone of strain Gsoil 1429^T was ubiquinone 10 (Q-10), in line with all other members of the family *Sphingomonadaceae*. The cellular fatty acids of strain Gsoil 1429^T and related type strains are listed in the Table 2.

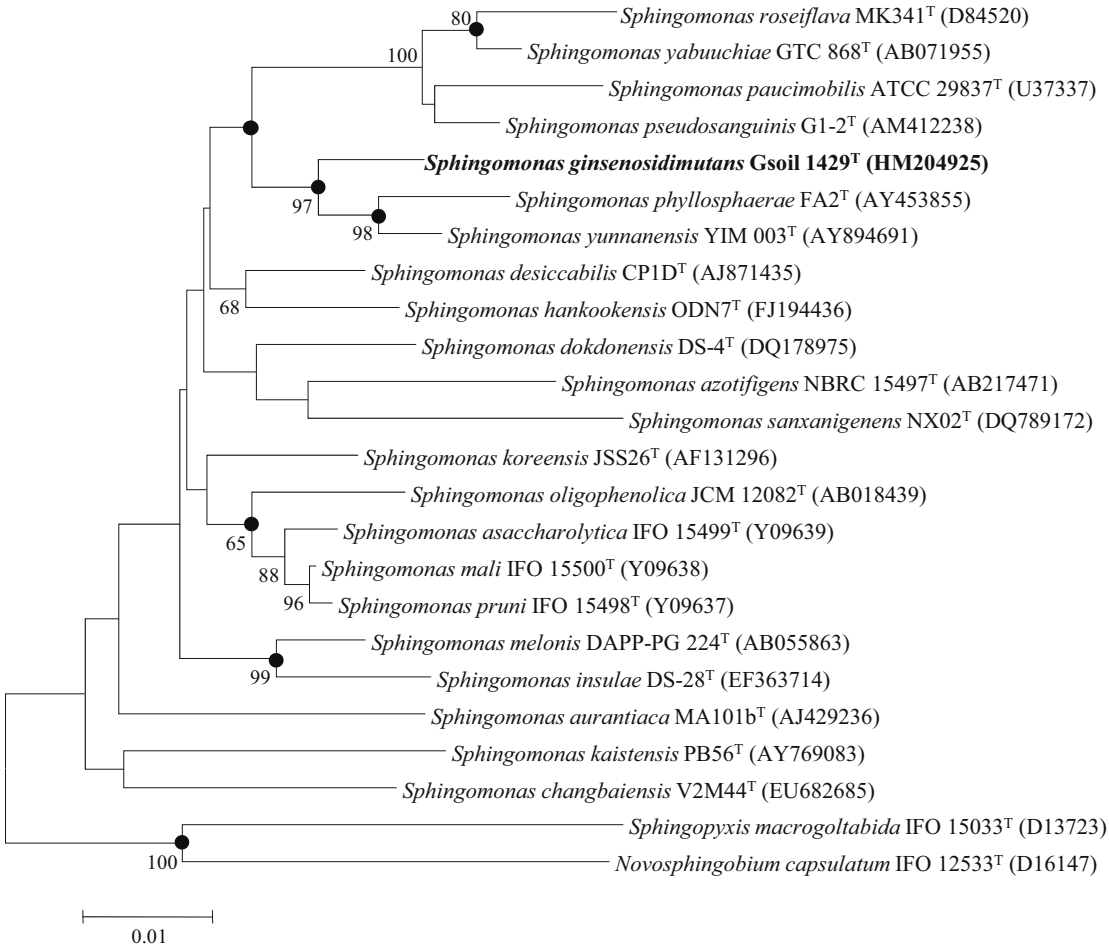

Fig. 2. Phylogenetic tree constructed from a comparative analysis of 16S rRNA gene sequences showing the relationships of *Sphingomonas ginsenosidimutans* Gsoil 1429^T with other related species. This tree was made using the neighbor-joining method (Saitou and Nei, 1987) with a Kimura (1983) two-parameter distance matrix and pairwise deletion. Dots indicate generic branches that were also recovered by using maximum-parsimony algorithms. Bootstrap values (expressed as percentages of 1,000 replications) greater than 65% are shown at the branch points. Bar, 0.01 substitutions per 1 nucleotide position.

Table 2. Cellular fatty acid profiles of strain Gsoil 1429^T and recognized *Sphingomonas* species.
Strains: 1, *Sphingomonas ginsenosidimutans* Gsoil 1429^T; 2, *Sphingomonas yunnanensis* YIM 003^T; 3, *Sphingomonas phyllosphaerae* FA2^T; 4, *Sphingomonas koreensis* JSS26^T; 5, *Sphingomonas asaccharolytica* IFO 15499^T.
All data from this study. All strain's fatty acids that account for less than 0.3% of the total fatty acids are not shown. Therefore, the percentages do not add up to 100%. -, not detected. Major fatty acids are shown in bold type.

Fatty acid	1	2	3	4	5
Saturated					
$C_{12:0}$	0.5	-	0.5	-	-
$C_{14:0}$	1.1	0.9	1.2	0.8	0.4
$C_{16:0}$	9.8	11.9	8.0	10.7	10.1
$C_{17:0}$	-	-	0.7	-	6.6
$C_{18:0}$	0.4	0.7	-	0.4	0.7
Unsaturated					
$C_{14:1}$ ω5c	3.9	3.5	6.3	3.3	2.2
$C_{16:1}$ ω5c	2.3	1.5	1.4	1.7	-
$C_{17:1}$ ω5c	-	-	-	-	0.5
$C_{17:1}$ ω6c	1.8	2.4	5.1	1.7	24.5
$C_{17:1}$ ω8c	-	0.5	0.9		3.4
$C_{18:1}$ ω5c	1.5	-	-	1.5	1.9
Branched-chain fatty acids					
iso-$C_{11:0}$ 3OH	-	0.3	1.0	-	-
iso-$C_{17:0}$ 3OH	2.3	2.2	3.9	2.0	1.4
iso-$C_{19:0}$	-	-	-	0.4	-
anteiso-$C_{15:0}$	2.2	1.9	3.6	1.7	1.2
anteiso-$C_{17:0}$	2.0	1.9	3.4	1.6	1.1
anteiso-$C_{17:1}$ ω9c	-	-	0.9	-	-
Hydroxy fatty acids					
$C_{14:0}$ 2OH	6.9	8.9	14.5	6.6	2.5
$C_{15:0}$ 2OH	-	-	1.4	-	3.7
$C_{16:0}$ 2OH	-	-		0.5	-
$C_{18:1}$ 2OH	-	1.1	1.4	-	-
Cyclo fatty acid					
$C_{19:0}$ cyclo ω8c	-	-	0.3	0.4	-
Summed feature[a]					
3; $C_{16:1}$ ω7c/$C_{16:1}$ ω6c	5.5	12.5	12.4	2.1	0.6
8; $C_{18:1}$ ω7c/ω9t/ω12t	58.0	45.7	30.4	59.6	31.0
Methyl ester					
$C_{18:1}$ ω7c 11-methyl	1.5	3.7	2.5	4.4	7.1

[a] Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed features consist of: 3, $C_{16:1}$ ω7c and/or $C_{16:1}$ ω6c; 8, $C_{18:1}$ ω7c and/or $C_{18:1}$ ω9t and/or $C_{18:1}$ ω12t.

The predominant fatty acid of all the 5 compared strains was $C_{18:1}$, which ranged from 30.4%-59.6% of the total fatty acids. According to the Table 2, qualitative and quantitative differences in fatty acid content could be observed between strain Gsoil 1429^T and its phylogenetically closest relatives. The polar lipids detected were phosphatidylglycerol, phosphatidylethanolamine, sphingoglycolipid, diphosphatidylglycerol, phosphatidylcholine, and an unknown lipid (Fig. 3).

DNA G+C content and DNA-DNA hybridization

The DNA G+C content of strain Gsoil 1429^T was 65.6 mol%, similar to those of *S. yunnanensis*, *S. phyllosphaerae*, *S. koreensis*, and *S. asaccharolytica*, which were in the range of 61-67.5 mol %. DNA-DNA relatedness values between strain Gsoil 1429^T and *S. yunnanensis* YIM 003^T, *S. phyllosphaerae* FA2^T, *S. koreensis* JSS26^T, and *S. asaccharolytica* IFO 15499^T were 28% (SD, 3.2%), 12% (SD, 1.5%), 15% (SD, 1.7%), and 8% (SD, 1.2%), respectively.

Taxonomic conclusions

The characteristics of strain Gsoil 1429^T were consistent with the description of the genus *Sphingomonas* with regard to morphological, biochemical and chemotaxonomic properties. However, the phylogenetic distance between strain Gsoil 1429^T and recognized *Sphingomonas* species, the unique phenotypic characteristics (Table 1) and the low level of DNA-DNA relatedness values (Wayne *et al*., 1987) warrant assignment of strain Gsoil 1429^T to the genus *Sphingomonas* as the type strain of a novel species, for which the name *Sphingomonas ginsenosidimutans* sp. nov. is proposed.

Description of *Sphingomonas ginsenosidimutans* sp. nov.

Sphingomonas ginsenosidimutans (gin.se.no.si.di.mu'tans. N.L. n. *ginsenosidum*, ginsenoside; L. part. adj. *mutans*, transforming, converting; N.L. part. adj. *ginsenosidimutans*, ginsenoside-converting)

Cells are Gram-reaction-negative, strictly aerobic, non-motile and non-spore-forming rods (0.3-0.5 μm in diameter and 1.2-1.8 μm in length) after culture on R2A agar for 2 days. Colonies are smooth, transparent, convex, circular with regular margins, yellowish in color, and 2-3 mm in diameter after two days on R2A agar. Growth also occurs on nutrient agar and TSA, but not on MacConkey. Grows on R2A at 18-42°C and at pH 6.0-10.0, but not at 10°C and 45°C. Optimum growth occurs at 25-30°C and at pH 7.0. Growth occurs well without NaCl supplement. Catalase and oxidase are positive. DNA, pullulan, laminarin are hydrolyzed. Does not hydrolyze casein and starch. Carbon assimilation tests as a sole carbon sources (API ID 32 GN, API 20 NE) and the enzyme activities (API ZYM) are listed in Table 1. Q-10 is the predominant respiretory quinone, and summed feature 8 (comprising $C_{18:1}$ ω7c/ω9t/ω12t), $C_{16:0}$ and $C_{14:0}$ 2OH are the major cellular fatty acids (>6%). The G + C content of the genomic DNA is 65.6 mol%. The polar lipids detected were phosphatidylglycerol, phosphatidylethanolamine, sphingoglycolipid, diphosphatidylglycerol, phosphatidylcholine, and an unknown lipid.

The type strain, Gsoil 1429^T (=KACC 14949^T =JCM 17074^T =LMG 25799^T) was isolated from soil of a ginseng cultivating field of Pocheon province, South Korea.

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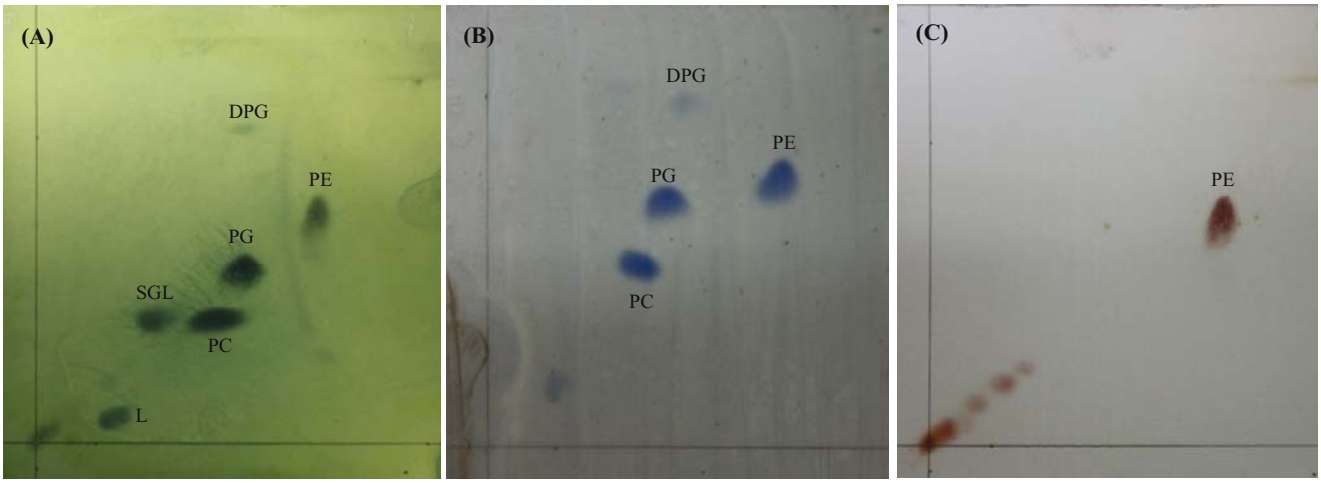

Fig. 3. Two-dimensional thin-layer chromatography of polar lipids of strain Gsoil 1429^T. Chloroform/methanol/water (65:25:4, by vol.) was used in the first direction, followed by chloroform/acetic acid/methanol/water 80:15:12:4, by vol.) in the second direction. The following spray reagents were used for detection: (A) 5% ethanolic molybdatophosphoric acid (for total lipids); (B) molybdenum blue (Sigma) (for phospholipids); (C) ninhydrin (for aminolipids). Abbreviations: PG, phosphatidylglycerol; PE, phosphatidylethanolamine; PC, phosphatidylcholine; SGL, sphingoglycolipid; DPG, diphosphatidylglycerol; L, an unknown lipid.

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